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Request for grant of a patent

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1.	**Your reference**	8.41.70236
2.	**Patent application number** *(The Patent Office will fill in this part)*	14 MAY 1999 **9911287.2**
3.	**Full name, address and postcode of the or of each applicant** *(underline all surnames)*	Affibody Technology Sweden AB c/o Nygren Singelgränd 36 SE-178 40 Ekerö Sweden
	Patents ADP number *(if you know it)*	76612830001 RdeS
	If the applicant is a corporate body, give country/state of incorporation	Sweden
4.	**Title of the invention**	Self-assembling Protein Structures
5.	**Name of your agent** *(if you have one)*	Frank B. Dehn & Co.
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	179 Queen Victoria Street London EC4V 4EL
	Patents ADP number *(if you know it)*	166001 ✓

6.	**If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and** *(if you know it)* **the or each application number**	**Country**	**Priority application number** *(if you know it)*	**Date of filing** *(day / month / year)*

7.	**If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**	**Number of earlier application**	**Date of filing** *(day / month / year)*

8.	**Is a statement of inventorship and of right to grant of a patent required in support of this request?** *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	Yes

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Description	7
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Priority documents	-
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
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Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. **I/We request the grant of a patent on the basis of this application.**

Signature **Date** 14 May 1999

Frank B. Dehn & Co.

12. **Name and daytime telephone number of person to contact in the United Kingdom**

J C Marsden
0171 206 0600

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SELF-ASSEMBLING PROTEIN STRUCTURES

This invention relates to self-assembling protein structures, more particularly to such structures formed using modular units capable of biospecific interaction.

The assembly of ordered macromolecular protein structures is well known in nature, for example in the construction of transcriptional complexes in eucaryotes and in mechanically stabilising structures such as collagen in bone. In such cases the principal interactions between the protein molecules comprise hydrogen bonding.

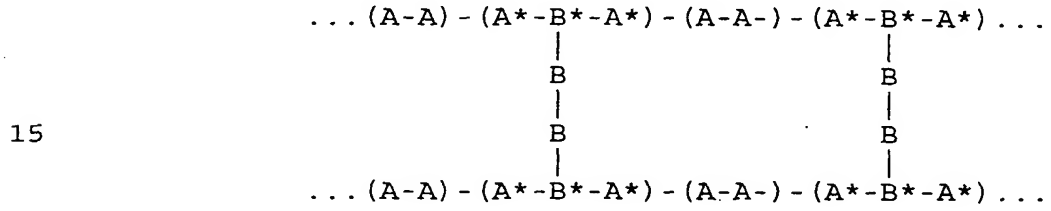
Peptides which contain alternating hydrophilic and hydrophobic amino acid residues and which are capable of spontaneous formation of macromolecular/macroscopic membranes are also known: see, for example, US-A-5670483. Such macroscopic materials, which may be held together by a combination of hydrophobic and ion-pair interactions, may be useful biomaterials for medical products such as slow release drug delivery vehicles, artificial sutures etc. It will be appreciated that the need to employ component molecules which are complementary and/or structurally compatible inevitably limits the application of such techniques.

The present invention is based on the finding that a wide variety of self-assembling protein structures may be formed using self-organising modular protein units which are capable of biospecific interactions. The various modular units, hereinafter referred to as affinity modules, comprise appropriate combinations of protein molecules selected to convey the required capability for biospecific interaction. Thus, for example, where A and A* represent a first pair of proteins having specific affinity for each other (a first affinity pair), and B and B* represent a second

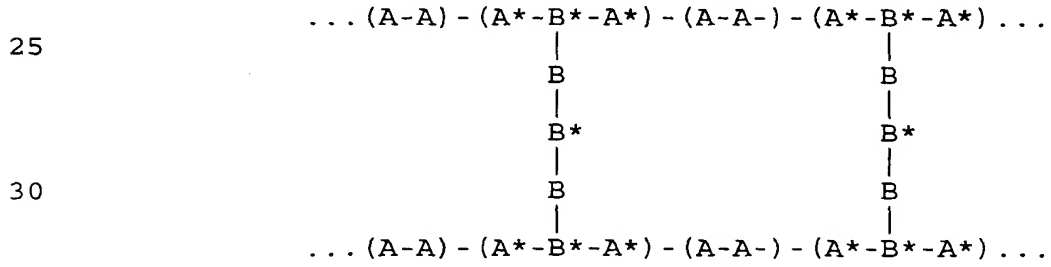
pair of proteins having specific affinity for each other (a second affinity pair), affinity modules A-A and A*-B*-A* may be used to create self-assembling linear structures of the type

...(A-A)-(A*-B*-A*)-(A-A-)-(A*-B*-A*)...

Additional use of B-B affinity modules may permit the assembly of sheet-like structures of the type



Three dimensional structures may similarly be assembled using an appropriate selection of affinity modules. Thus, for example, A-A, A*-B*-A* and B-B*-B modules may self-assemble to form sheets of the type



Two such sheets may be linked together by using B-B modules to bind the B* moieties in the B-B*-B linking groups of adjacent sheets, so as to form a two-sheet structure.

It will be appreciated from the foregoing that by using two or more affinity pairs of proteins (which term as used herein with regard to the invention also embraces appropriate peptides) which have specific and exclusive affinity for each other, by selecting

appropriately constructed affinity modules in appropriate relative amounts, and where necessary by interacting the affinity modules in an appropriate order, it is possible to form self-assembled protein structures in a highly specific, predictable and controllable manner and with widely ranging degrees of complexity.

Thus according to one aspect of the invention there is provided a self-assembled protein structure comprising at least two pairs of proteins wherein the proteins of each of said pairs have specific and exclusive affinity for each other, said proteins being linked to form affinity modules capable of mutual biospecific interaction to form an assembled structure.

The aforesaid affinity modules, which themselves constitute a feature of the invention, may for example comprise naturally small and highly soluble protein domains, such as individual domains of the bacterial surface receptors of staphylococcal protein A (SPA) or streptococcal protein G (SPG) (Ståhl, S. and Nygren, P-Å., Patologie Biologie **45**, pp. 66-76 [1997]; Jansson, B. *et al.*, FEMS Microbiology and Immunology **20**, pp. 69-78 [1998]). Domains having affinity therefor may be found in collections or libraries of individual domains of SPA (Nord, K. *et al.*, Nature Biotechnology **15**, pp. 772-777 [1997]), SPG, antibody fragments such as single-chain Fv, Fv or Fab-fragments (Hoogenboom *et al.*, Immunotechnology **4**, pp. 1-20 [1998]), other protein domains (Nygren, P.-Å. and Uhlén, M., Curr. Opin. Struct. Biol. **7**, pp. 463-469 [1997]) or linear or cyclic peptides (Cortese, R. *et al.*, Curr. Opin. Biotechnol. **6**, pp. 73-80 [1995]), for example created using combinatorial protein technology; affinity pairs may also be found from such collections or libraries. Identification of variants having the desired binding capabilities may be performed using standard *in vitro* selection techniques such as phage display technology

(Clackson, T. and Wells, J., Trends Biotechnol. **12**, pp. 173-184 [1994]; Smith, G.P. and Petrenko, V.A., Chem. Rev. **97**, pp. 391-410 [1997]), ribosomal display (Hanes, J. and Plückthun, A., Proc. Natl. Acad. Sci. U.S.A. **94**, pp. 4937-4942 [1997]), peptides on plasmids (Schatz, P.J., Bio/technol. **11**, pp. 1138-1143 [1993]) or mRNA-protein coupling using pyromycin (Roberts, R.W. and Szostak, J.W., Proc. Natl. Acad. Sci. U.S.A. **94**, pp. 12297-12302 [1997]).

The affinity modules may be synthesised by recombinant DNA technology, by transformation of a suitable host cell with a plasmid or viral construct encoding for the affinity module protein, or by chemical synthesis.

Chemical or genetic fusion may be employed as necessary to link protein molecules so as to form appropriate bi- or multi-valent or bi- or multi-functional affinity modules.

Typically, the affinity between modules should be in the range of $K_{aff} = 10^4$-10^{11} M^{-1} in order to allow stable self-assembled structures to form under a range of conditions, for example under physiological conditions or at different pHs and/or temperatures. For certain applications it may, however, be advantageous to prepare structures which may be reversibly disassembled as a result of, for example, pH or temperature change.

The nature of self-assembled protein structures formed in accordance with the invention may, for example, be investigated by techniques such as size exclusion chromatography, electron microscopy, viscosity analyses, microcalorimetry, nuclear magnetic resonance spectroscopy and X-ray crystallography.

Chemical groups and/or molecules (including further proteins) may if desired be incorporated into the affinity modules, for example by chemical coupling or genetic fusion, thereby permitting the preparation of assembled protein structures with a variety of

properties and functionalities. Protein structures obtainable in accordance with the invention may thus have a wide range of applications, including, for example diagnostic applications, e.g. to obtain highly avid reagents; clinical applications, e.g. to obtain controlled delivery of therapeutics; nano-fabrication applications, e.g. to obtain spontaneous build up of ordered small-scale structures; biotechnological applications, e.g. to obtain thermally or chemically reversible protein networks; and provision of basis for stepwise enzymatic treatment of a substrate at a defined position.

Thus, for example, in the formation of highly avid diagnostic reagents, effector molecules such as reporter enzymes or other detection molecules, including alkaline phosphatase, β-galactosidase, horse radish peroxidase, fluourescein isothiocyanate, green fluorescent protein or derivatives thereof, luciferase and/or additional affinity reagents such as biotin, streptavidin, cellulose binding domains, zinc fingers, antibody fragments or protein or peptide moieties may be chemically or genetically coupled to a suitable affinity module.

In clinical applications enzymes or therapeutics (including vaccines) may be encapsulated in self-assembling protein structures by mixing the affinity modules with the substance to be encapsulated. Controlled release of the active substance may occur upon, for example, a change in buffer or temperature conditions, or *in vivo* upon spontaneous disassembling of the structures. An advantage of the *in vivo* use of self-assembling protein structures is that these are normally biodegradable.

Self-assembling protein systems in accordance with the invention may be used to provide controlled "plugging" of cavities or holes, either *ex vivo* or *in vivo*, for example by controlled release of enzymes or

other active substances. Thus, for example, protein self-assembly systems capable of preventing bleeding may be prepared by derivatisation of affinity modules with substances directing the assembly to specific sites around injuries.

Self-assembled protein structures according to the invention may be used in the preparation of hydrogels capable of reversible gelation under selected buffer or temperature conditions, and in the preparation of protein fibres or films which may be used to form biodegradable materials such as threads or cloths.

Following address-specific deposition of a suitable anchoring affinity module onto a bio-chip, higher forms of structures may be produced in a stepwise manner using further affinity modules in accordance with the invention. Such self-assembly may be used to create structures for use in the detection of or functional studies of biological samples. In addition, through derivatisation of affinity modules with suitable substances such as enzymes or organic substances, a build up of complexes mimicking natural systems, for example substrate conversion by concerted action of several enzymes, or production of energy or light as in photosynthesis or photorespiration, may be accomplished.

In bioelectronic applications the spontaneous assembly of affinity modules into ordered structures may, for example, serve as a means to create small circuits useful for conducting electric charges or currents. Suitably derivatised affinity modules, e.g. highly charged modules or modules into which molecular groups with high electron densities have been directly or indirectly incorporated, may be used to prepare self-assembled structures useful as "wires" for nano-fabrication of electric circuit components such as resistors, transistors or capacitors. Self-assembled structures may also be used for site-specific deposition of metallic materials such as silver or gold, for

example in order to create anchoring points from which a circuit may be built up by attachment of appropriate affinity modules, for example using affinity modules containing cysteine residues to couple to deposited gold.

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